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Plasma/Serum HIV RT-PCR Detection Kit — Product Insert

Product # 33800

Human immunodeficiency virus (HIV) is a lentivirus (a member of the retrovirus family) that causes acquired immunodeficiency syndrome (AIDS), a condition in humans in which the immune system begins to fail, leading to life-threatening opportunistic infections. Infection with HIV occurs by the transfer of blood, semen, vaginal fluid, pre-ejaculate, or breast milk. Within these bodily fluids, HIV is present as both free virus particles and virus within infected immune cells. The four major routes of transmission are unsafe sex, contaminated needles, breast milk, and transmission from an infected mother to her baby at birth (vertical transmission). Screening of blood products for HIV has largely eliminated transmission through blood transfusions or infected blood products in the developed world. HIV infection in humans is considered pandemic by the World Health Organization (WHO). From its discovery in 1981 to 2006, AIDS has killed more than 25 million people. HIV infects about 0.6% of the world's population. In 2005 alone, AIDS claimed an estimated 2.4–3.3 million lives, of which more than 570,000 were children. HIV infects primarily vital cells in the human immune system such as helper T cells (CD4+ T cells), macrophages, and dendritic cells. HIV progresses to AIDS at a variable rate affected by viral, host, and environmental factors; HIV-specific treatment delays this process. Most will progress to AIDS within 10 years of HIV infection: some will have progressed much sooner and some will take much longer. Treatment with anti-retrovirals increases the life expectancy of people infected with HIV. Even after HIV has progressed to diagnosable AIDS, the average survival time with antiretroviral therapy was estimated to be more than 5 years as of 2005. Without antiretroviral therapy, someone who has AIDS typically dies within a year.

Principle of the Test

Norgen's Plasma/Serum HIV RT-PCR Detection Kit constituents a ready-to-use system for the isolation and detection of HIV viral RNA using end-point RT-PCR. The kit first allows for the isolation of circulating RNA, including viral RNA, from the Plasma/Serum samples using Norgen's proprietary resin as the separation matrix. The viral RNA is isolated free from inhibitors, and can then be used as the template in a RT-PCR reaction for HIV detection using the provided HIV Master Mix. The HIV Master Mix contains reagents and enzymes for the specific amplification of a 297 bp region of HIV. In addition, Norgen's Plasma/Serum HIV RT-PCR Detection Kit contains a second Mastermix, the Control RT-PCR Master Mix, which can be used to identify possible PCR inhibition and/or inadequate isolation via a separate PCR reaction with the use of the *PCR control (PCRC)* or *Isolation Control (IsoC),* respectively. The amplification and detection of either the *HIV Isolation Control (IsoC)* or the *PCR control (PCRC)* does not reduce the detection limit of the analytical HIV PCR. The kit is designed to allow for the testing of 24 samples.

Kit Components:

Component	Contents
PS Solution A	6 mL
PS Solution B	45 mL
PS Solution C	9 mL
Wash Solution	22 mL
RNA Elution Solution	3 mL
Mini Filter spin Columns	24
Collection Tubes	24
Elution tubes (1.7 mL)	24
HIV 2x RT-PCR Master Mix	**0..35 mL**
Control 2x RT-PCR Mastermix	**0.35 mL**
***Isolation Control (IsoC)*[a]**	**0.3 mL**
***HIV Positive Control (PosC)*[b]**	**0.1 mL**
Nuclease Free-Water	1.25 mL
Norgen's DNA Marker	0.1 mL
Product Insert	1

* *IsoC = Isolation Control ; PosC= Positive Control*

[a] *The isolation control is a RNA transcript product.* [b] *The positive control is HIV RNA transcript*

Customer-Supplied Reagents and Equipment

- Centrifuge with a swinging bucket rotor capable of 2000 RPM
- Benchtop microcentrifuge
- Micropipettors
- 96 – 100% ethanol
- β - Mercaptoethanol
- 15 mL tubes
- Disposable Latex gloves
- Micropipettes with an accuracy range between 1-10 µL, 10-100 µL and 100-1000 µL
- Laminar flow hood for extractions
- Vortex
- Sterile, nuclease-free aerosol-barrier micropipettor tips
- Microcentrifuge tube rack
- PCR tubes

Storage Conditions and Product Stability

- All buffers should be kept tightly sealed and stored at room temperature (15-25oC) for up to 1 year without showing any reduction in performance.
- It is recommended to warm PS Solution A, PS Solution B and PS Solution C for 20 minutes at 60oC if any salt precipitation is observed.
- The Positive Control (***HIV PosC,*** red cap) and Isolation Control (***IsoC*****,** orange cap) should be stored at -70°C. If needed, make aliquots of the controls according to the volume used in the protocol (10 µL of ***HIV PosC*** or 15 µL of ***IsoC***) prior to freezing.
- The HIV 2X RT-PCR Mastermix and the Control 2X RT-PCR Mastermix should be stored at -20°C. Make appropriate aliquots if needed
- All other kit components may be stored at room temperature
- The HIV 2X RT-PCR Mastermix, the Control 2X RT-PCR Mastermix, HIV Postive Control (PosC) and the Isolation Control (IsoC) should not undergo repeated freeze-thaw (a maximum freeze-thaw of three times).
- Allow reagents to thaw at room temperature prior to use
- After addition of samples to RT-PCR Master Mix use within one hour
- Kit reagents are stable through the end of the expiration month indicated on the packaging label when stored at the recommended temperatures.

General Precautions

- Follow universal precautions. All patient specimens should be considered as potentially infectious and handled accordingly.
- Diagnostic laboratory work on clinical samples from patients who are suspected of having HIV infection should be conducted in a BSL2 laboratory. All sample manipulations should be carried out in a biosafety cabinet. Viral isolation on clinical specimens from patients who are suspected of having HIV infection should be performed in a BSL2 laboratory with BSL3 practices
- Wear personal protective equipment, including gloves and lab coats when handling kit reagents. Wash hands thoroughly when finished performing the test.
- Do not smoke, drink or eat in areas where kit reagents and/or human specimens are being used.
- Dispose of unused kit reagents and human specimens according to local, provincial or federal regulations.
- Workflow in the laboratory should proceed in a uni-directional manner, beginning in the pre-amplification area(s) (i.e. specimen collection and RNA extraction) and moving to the amplification / detection area(s) (RT-PCR and gel electrophoresis).
- Do not use supplies and equipment across the dedicated areas of specimen extraction and sample preparation. No cross-movement should be allowed between the different areas.
- Supplies and equipment used for specimen preparation should not be used for pipetting or processing amplified RNA or other sources of target nucleic acids.
- All amplification supplies and equipment should be kept in the amplification / detection area at all times.
- Personal protective equipment, such as laboratory coats and disposable gloves, should be area specific.
- As contamination of patient specimens or reagents can produce erroneous results, it is essential to use aseptic techniques.
- Pipette and handle reagents carefully to avoid mixing of the samples.

- Use proper pipetting techniques and maintain the same pipetting pattern throughout the procedure to ensure optimal and reproducible values.
- Do not substitute or mix reagents from different kit lots or from other manufacturers.
- Do not interchange reagent tube / bottle caps as this may lead to contamination and compromise test results.
- Only use the protocol provided in this insert. Alterations to the protocol and deviations from the times and temperatures specified may lead to erroneous results.

Quality Control
In accordance with Norgen's ISO 9001 and ISO 13485-certified Quality Management System, each lot of Norgen's HIV 2x RT-PCR Master Mix, Control 2X RT-PCR Mastermix, Isolation Control (IsoC) and the *HIV Positive Control* (*PosC*) are tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations
Norgen's Plasma/Serum HIV RT-PCR Detection Kit is designed for research purposes only.

Product Warranty and Satisfaction Guarantee
NORGEN BIOTEK CORPORATION guarantees the performance of all products in the manner described in our product manual. The customer must determine the suitability of the product for its particular use.

Safety Information
Biosafety level 2 practices are recommended for works involving clinical samples from patients who are suspected of having HIV infection. Ensure the appropriate containment equipment and facilities are used for activities involving cultures or potentially infectious clinical materials. Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These are available as convenient PDF files online at www.norgenbiotek.com.

CAUTION: DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

PS Solution A, **PS Solution B** and **PS Solution C** contain guanidinium salts, and should be handled with care. Guanidinium salts form highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions

If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

Plasma or serum of all human and animal subjects is considered potentially infectious. All necessary precautions recommended by the appropriate authorities in the country of use should be taken when working with plasma or serum

1. Protocol

A. Specimen Collection, Storage and Transport

Precaution: All samples have to be treated as potentially infectious material.

1. Specimen Collection and Sample Storage
- Blood withdrawal causes injury of blood vessels (arteries, veins and capillaries).
- Only safe and sterile material should be used.
- For blood withdrawal appropriate disposables are available. For the vein puncture, too fine capillary needles should not be employed.
- Venous blood withdrawal should be carried out on the appropriate parts of the elbow bend, the forearm or the back of the hand.
- Blood has to be withdrawn with standard specimen collection tubes (red cap, Sarstedt or equivalent tube of another manufacturer). 5 - 10 ml EDTA blood should be withdrawn.

Precaution: Samples of heparinised humans must not be used

2. Sample Storage

- Whole blood should be separated into plasma and cellular components by centrifugation for 20 minutes at 800 - 1,600 x g within six hours. The isolated plasma has to be transferred into sterile polypropylene tubes.
- The sensitivity of the assay can be reduced if you freeze the samples as a matter of routine or store them for a longer period of time.
- Virus encapsulated RNA is stable for weeks if stored at -20°C and even for months and years when stored at -70°C.

3. Sample Transport

- Sample material should be transported in a shatterproof, leak-proof transport container as a matter of principle. Thus, a potential danger of infection due to a leakage of sample can be avoided.
- The samples should be transported following the local and national instructions for the transport of pathogen material.
- We recommend sample transport with a courier. The blood samples should be shipped cooled (+2°C to +8°C) and the separated plasma deep frozen (-20°C).

4. Interfering substances

- Elevated levels of bilirubin (15 mg/dl) and lipids (800 mg/dl) and haemolytic samples do not influence the system.
- Heparin (10 IU/ml) affects the PCR. Samples which have been collected in tubes containing heparin as an anticoagulant should not to be used. Also, samples of heparinised patients must not be used.

B. Purification of HIV RNA from 1mL Serum or Plasma

Working with RNA

RNases are very stable and robust enzymes that degrade RNA. Autoclaving solutions and glassware is not always sufficient to actively remove these enzymes. The first step when preparing to work with RNA is to create an RNase-free environment. The following precautions are recommended as your best defence against these enzymes.

- The RNA area should be located away from microbiological work stations
- Clean, disposable gloves should be worn at all times when handling reagents, samples, pipettes, disposable tubes, etc. It is recommended that gloves are changed frequently to avoid contamination
- There should be designated solutions, tips, tubes, lab coats, pipettes, etc. for RNA only
- All RNA solutions should be prepared using at least 0.05% DEPC-treated autoclaved water or molecular biology grade nuclease-free water
- Clean all surfaces with commercially available RNase decontamination solutions
- When working with purified RNA samples, ensure that they remain on ice during downstream applications

Notes Prior to Use

- All centrifugation steps are performed at room temperature.
- A variable speed centrifuge should be used for maximum kit performance. If a variable speed centrifuge is not available a fixed speed centrifuge can be used, however reduced yields may be observed.
- Ensure that all solutions are at room temperature prior to use.
- Prepare a working concentration of the **Wash Solution** by adding 50 mL of 96 - 100% ethanol (provided by the user) to the supplied bottle containing the concentrated RNA Wash Solution. This will give a final volume of 72 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- The use of β-mercaptoethanol in lysis is highly recommended to isolate RNA for sensitive downstream applications. Add 10 µL of β-mercaptoethanol (provided by the user) to each 1 mL of PS Solution B..
- ***PS Solution A contains resin and must be mixed well before every pipetting.***
- It is highly recommended to warm up **PS Solution A**, **PS Solution B** and **PS Solution C** at 60^{o}C for 20 minutes and mix well until the solutions become clear again if precipitates are present.
- It is important to work quickly during this procedure.
- ***This kit is suitable for the isolation of Viral RNA from serum or plasma prepared from blood collected on either EDTA or citrate. Plasma samples prepared from blood collected on heparin***

should not be used as heparin can significantly interfere with many downstream applications such as RT-PCR.

- It is important to work quickly during this procedure.
- An *Isolation Control* (*IsoC*) is supplied. This allows the user to control the viral RNA isolation procedure. For this assay, add the *Isolation Control* (*IsoC*) to the lysate during the isolation procedure.
    - The *Isolation Control* (*IsoC*) **must not** be added to the sample material directly
    - Do not freeze and thaw the *Isolation Control* (*IsoC*) more than 3 times.
    - The *Isolation Control* (*IsoC*) must be kept on ice at all times during the isolation procedure.

1. In a 50 mL tube (provided by the user), add 0.2 mL of **PS Solution A** and 1.8 mL **PS Solution B** (after the addition of β-mercaptoethanol) to 1 mL plasma/serum sample. Mix well by vortexing for 15 seconds. ***(Note: PS Solution A contains resin and must be mixed well before every pipeting)***
2. Incubate the mixture from **Step 1** for 10 minutes at 60^{o}C.
3. After incubation add 3 mL of 96-100% ethanol (provided by the user). Mix well by vortexing for 15 seconds.
4. Centrifuge for **30 seconds at 1,000 RPM**, then carefully decant the supernatant in order to ensure that the slurry pellet is not dislodged.
5. To the slurry pellet add 0.3 mL **PS Solution C**, and mix well by vortexing for 15 seconds.
6. Incubate the mixture from **Step 5** for 10 minutes at 60^{o}C.
7. After incubation add 0.3 mL 96-100% ethanol (provided by the user) then add 10 µL of **Isolation Control (*IsoC*)** to the lysate mixture. Mix well by vortexing for 15 seconds.
8. Transfer 650 µL from the mixture from **Step 7** into a Mini Filter Spin column. Centrifuge for **1 minute at 14,000 RPM**. Discard the flowthrough and reassemble the spin column with its collection tube.
9. Repeat Step 8 until all the mixture from **Step 7** has been transferred to the Mini Filter Spin column.
10. Apply 400 µL of **Wash Solution** to the column and centrifuge for **1 minute at 14,000 RPM**. Discard the flowthrough and reassemble the spin column with its collection tube.
11. Repeat step 10 two more times, for a total of three washes.
12. Spin the column, empty, for **3 minutes at 14,000 RPM**. Discard the collection tube.
13. Transfer the spin column to a fresh 1.7 mL Elution tube. Apply 100 µL of **Elution solution** to the column and centrifuge for **2 minutes at 2,000 RPM,** followed by **3 minute at 14,000 RPM**.

C. HIV RT-PCR Assay Preparation

Notes:

- Before use, suitable amounts of all RT-PCR components should be completely thawed at room temperature, gently vortexed and centrifuged briefly.
- The amount of HIV 2X RT-PCR Master Mix provided is enough for up to 32 RT-PCR reactions (24 sample RT-PCR, 4 positive control RT-PCR and 4 no template control RT-PCR).
- For each sample, one RT-PCR reaction using the **HIV 2X RT-PCR Mastermix** and one RT-PCR reaction using **Control 2X RT-PCR Mastermix** should be set up in order to have a proper interpretation of the results.
- For every RT-PCR run, one reaction containing HIV Positive Control (**HIV PosC**) and one reaction as no template control (*Nuclease Free-Water*) must be included for proper interpretation of results.
- The recommended minimum number of RNA samples tested per RT-PCR run is 6.
- Using a lower volume from the sample than recommended may affect the sensitivity of HIV Limit of Detection.

1. Prepare the RT-PCR reaction for sample detection (Set #1, using **HIV 2X Detection RT-PCR Mastermix**) and the RT-PCR reaction for control detection (Set #2, using **Control 2X RT-PCR Mastermix**) as shown in Table 1 below. The recommended amount of sample RNA to be used is 1 - 2 µL. Ensure that one HIV detection reaction and one control reaction is prepared for each RNA sample. Adjust the final volume of the RT-PCR reaction to 20 µL using the Nuclease-Free Water provided.

Table 1. RT-PCR Assay Preparation

RT-PCR Components	Volume Per RT-PCR Reaction
HIV 2X RT-PCR Master Mix Or Control 2X RT-PCR Master Mix	**10 µL**
Sample RNA	**2 µL**
Nuclease-Free Water	**8 µL**
Total Volume	**20 µL**

2. For every RT-PCR run, prepare **one** positive control RT-PCR as shown in Table 2 below:

Table 2. RT-PCR Positive Control Preparation

RT-PCR Components	Volume Per RT- PCR Reaction
HIV 2X RT-PCR Master Mix Or Control 2X RT-PCR Master Mix	**10 µL**
HIV *Positive Control (PosC)*	**10 µL**
Total Volume	**20 µL**

3. For every RT- PCR run, prepare **one** no template control RT-PCR as shown in Table 3 below:

Table 3. RT-PCR Negative Control Preparation

RT-PCR Components	Volume Per RT-PCR Reaction
HIV 2X RT- PCR Master Mix Or Control 2X RT-PCR Master Mix	**10 µL**
Nuclease-Free Water	**10 µL**
Total Volume	**20 µL**

Therefore, at a minimum, each PCR run will contain 6 separate RT-PCR reactions.

C. HIV One Step RT-PCR Assay Programming

1. Program the thermocylcer according to the program shown in Table 4 below.
2. Run one step RT-PCR

Table 4. HIV Assay Program

One Step RT-PCR Cycle	Step	Temperature	Duration
Cycle 1	Step 1	50°C	30 min
Cycle 2	Step 1	95°C	3 min
Cycle 3 (40x)	Step 1	94°C	15 sec
	Step 2	60°C	30 sec
	Step 3	72°C	45 sec
Cycle 4	Step 1	72°C	5 min
Cycle 5	Step 1	4°C	∞

D. HIV One Step RT-PCR Assay Results Interpretation

1. For the analysis of the RT-PCR data, the entire 20 µL RT-PCR Reaction should be loaded on a 1X TAE 1.5% Agarose RNA gel along with 10 µL of Norgen's RNA Marker (provided).
2. The RT-PCR products should be resolved on the 1X TAE 1.5% Agarose gel at 150V for 20 minutes (Gel running time will be vary depending on an electrophoresis apparatus).
3. Sample results are provided below:

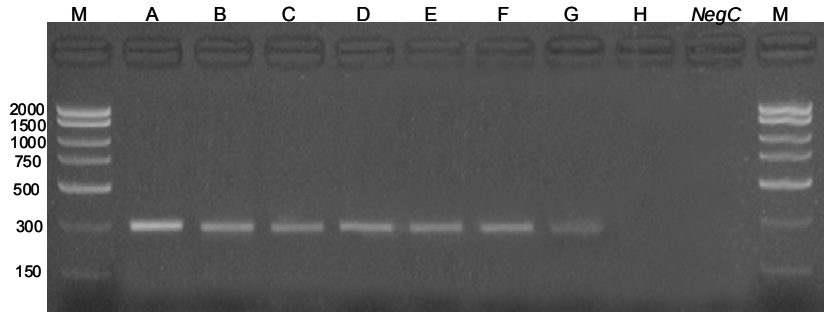

Figure 1: A representative 1X TAE, 1.7% agarose gel showing the amplification of HIV at different concentrations (*Target*). The size of the HIV target amplicon corresponds to the 297 bp band represented by the provided DNA Marker (M). Lanes A-H represents samples spiked with different HIV concentrations isolated from 2mL plasma samples (interpreted as positive results). The HIV spiked in plasma samples is an in vitro transcribed HIV RNA fragments.

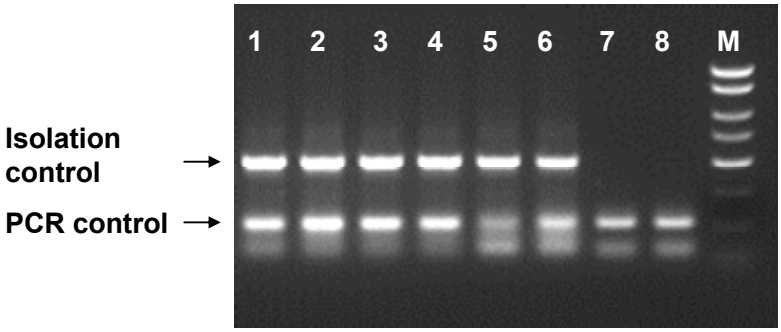

Figure 2: A representative 1X TAE 1.5% agarose gel showing the amplification of **Isolation Control** and **PCR Control** under different conditions using the **Control 2X RT-PCR Mastermix**. The size of the Isolation Control amplicon and PCR Control amplicon correspond to 499 bp and 150 bp, respectively, as represented by the provided DNA Marker (M). Lanes 1 to 6 showed detection of both Isolation Control and PCR Control, suggesting that the RNA isolation as well as the RT-PCR reaction was successful. Lane 7 and 8 showed only the detection of PCR Control suggesting that while the RT-PCR was successful, the isolation failed to recover even the spiked-in Isolation control.

Table 5. Interpretation of One Step RT-PCR Assay Results

Input Type	Target reaction	Control Reaction		Interpretation
	HIV Target Band (297 bp)	*IsoC* Band (499 bp)	*PCRC* Band (150 bp)	
Positive Control	X	X	X	Valid
Negative Control			X	Valid
Sample	X	X	X	Positive
Sample		X	X	Negative
Sample			X	Re-test
Sample				Re-test
Sample		X		Negative
Sample	X		X	Positive
Sample	X	X		Positive
Sample	X			Re-test

****** For results obtained that are not covered in Table 5 above, please refer to the Troubleshooting Section.

E. Specificity

The specificity of Norgen's Plasma/Serum HIV RT-PCR Detection Kit is first and foremost ensured by the selection of the HIV-specific primers, as well as the selection of stringent reaction conditions. The primers were checked for possible homologies in GenBank published sequences by sequence comparison analyses. Furthermore, the specificity of the HIV-specific primers were tested against most of the known sexually-transmitted pathogens.

F. Linear Range

- The linear range of Norgen's Plasma/Serum HIV RT-PCR Detection Kit was determined by analyzing a dilution series of HIV quantitative standards ranging from 8.46 x 10^9 VP/µl to 1 x 10^{-1} IU/µl.
- Each dilution has been tested in replicates (n = 4) using Norgen's Plasma/Serum HIV RT-PCR Detection Kit on 1X TAE, 1.7% Agarose gels.
- The linear range of Norgen's Plasma/Serum HIV RT-PCR Detection Kit has been determined to cover concentrations from 10 VP/µl to at least 8 x 10^6 VP/µl
- Under the conditions of Norgen's Plasma/Serum circulating RNA Isolation procedure, Norgen's Plasma/Serum HIV RT-PCR detection Kit covers a linear range from 1000VP/mL Plasma/Serum to at least 8 x 10^9 VP/mL Plasma/Serum.

G. Frequently Asked Questions

1. How many samples should be included per PCR run?

- Norgen's Plasma/Serum HIV RT-PCR Detection Kit is designed to test 24 samples. For every 6 samples, a Negative Control and a Positive Control must be included. It is preferable to pool and test 6 samples at a time. If not, the provided Negative Control and Positive Control are enough to run 3 samples at a time.

2. How can I interpret my results for a sample if neither the HIV PCR control nor the HIV Isolation Control (IsoC) amplifies?

- If neither the HIV PCR control nor the HIV Isolation Control (IsoC) amplifies, the sample must be re-tested. If the positive control showed amplification, then the problem occurred during the isolation, where as if the Positive control did not amplify the problem has occurred during the setup of the PCR assay reaction.

3. How should it be interpreted if only the HIV RT-PCR control showed amplification but neither the HIV target nor the HIV Isolation Control (IsoC) amplified for a sample?

- This indicates a poor isolation. The isolation procedure must be repeated.

4. How should it be interpreted if only the HIV Isolation Control (IsoC) was amplified in a sample?

- The sample tested can be considered as HIV negative.

5. How should it be interpreted if only the HIV target and the HIV RT-PCR control were amplified in a sample?

- The sample tested can be considered as HIV positive.

6. How should it be interpreted if only the HIV target was amplified in a sample?

- The sample tested can be considered positive. At high HIV viral load, the HIV amplicon will be predominant and the HIV PCR control as well as the HIV Isolation control may not amplify.

7. How should it be interpreted if only the HIV RT-PCR control and the HIV Isolation Control (IsoC) showed amplification?

- The sample tested can be considered negative

8. Can I process a different Plasma/Serum volume?

- The reagents provided with the isolation kit are only sufficient to process 24 Plasma/Serum samples of 2mL each.

9. What If I added more or less of the specified reagents' volume during RNA isolation?

- Adding less volume may reduce your RNA yields. Adding more may not affect the RNA yields EXCEPT if more Elution Buffer was added. Eluting RNA in higher volumes of Elution Buffer will result in diluting your RNA.

10. What If I forgot to do a dry spin after my second wash?

- Your RNA elution will be contaminated with the Wash Solution. This may dilute the RNA yield in your elution and it may interfere with your down stream applications.

11. What If I forgot to add the HIV Isolation control during the Isolation?

- The Isolation must be repeated.

Technical Assistance

NORGEN's Technical Service Department is staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of NORGEN products. If you have any questions or experience any difficulties regarding Norgen's Plasma/Serum HIV PCR Detection Kit or NORGEN products in general, please do not hesitate to contact us.

NORGEN customers are a valuable source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at NORGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362 or call one of the NORGEN local distributors (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

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